American Journal of Medical Genetics 68:338–341 (1997)

Abstracts From the 2nd NICHD Conference on the RSH/SLO Syndrome

John M. Opitz

Foundation for Developmental and Medical Genetics, Helena, Montana

1

COMPARISON OF PSYCHOMOTOR DEVELOPMENT IN EARLY-TREATED AND LATER-TREATED SIBS WITH SMITH-LEMLI-OPITZ SYNDROME. D.N. Abuelo. Genetic Counseling Center, Rhode Island Hospital and Brown University School of Medicine, Providence, Rhode Island.

Since the discovery of the metabolic defect in Smith-Lemli-Opitz Syndrome (SLOS), treatment protocols have been instituted using dietary supplements of cholesterol with or without the addition of bile acids. Serum cholesterol levels have increased and, in some patients, parents and teachers have noted improvement in behavior and development. Since most children identified as having SLOS are being offered dietary treatment, randomized controlled studies to evaluate objectively results of treatment cannot be performed. I have treated 2 sibs with cholesterol, one since age 2½ years, the other, who was identified prenatally [Abuelo et al., Am J Med Genet 56:281, 1995] since shortly after birth, and have attempted to compare their biochemical and developmental responses.

Past and recent studies of sibs with SLOS [Bialer et al., Am J Med Genet 28:723–731, 1987; Cunniff et al., Proc Greenwood Genet Center, in press] have shown that they are generally concordant for clinical findings and overall severity. This pair of sibs had pretreatment cholesterol levels, including cord blood in the younger sib, between 18 and 33 mg/dl. Cholesterol supplementation began at 50 mg/kg/day and was gradually increased to 100 mg/kg/day, resulting in an increase in their cholesterol levels to 60 to 80 mg/dl. The older sib had operations during the first year of life for cleft palate, undescended testes and metopic craniosynostosis. At 3 years, he was found to have bilateral sensorineural hearing loss. The younger sib has a bicuspid aortic valve. Both have been growing below the third centiles for weight and head circumference. Their psychomotor development has been monitored at periodic intervals by their early intervention programs, including assessments of gross and fine motor skills, cognition and communication. Results of testing between the chronologic ages of 18 to 24 months showed functioning between the 6- and 12-month levels.

The younger sib exhibited significant developmental delay despite the institution of early treatment. She performed slightly better on the fine and gross motor subtests, but the most striking differences were in tests of receptive and expressive language, most likely due to the deafness in the older sib. Thus, early dietary supplementation using the present protocol was not able to prevent the mental retardation associated with SLOS, although there may have been some amelioration. Longer-term studies of these and other sibs will have to be carried out before any conclusions can be drawn with regard to whether early treatment may change the course of the developmental delay in SLOS. All patients should receive systematic developmental testing so that we may evaluate both present and potential therapies.

2

ABNORMAL CHOLESTEROL BIOSYNTHESIS IN THE SMITH-LEMLI-OPTIZ AND THE LETHAL ACRODYSGENITAL SYNDROMES. V. Cormier-Daire, C. Wolf, M. Le Merrer, A. Nivelon, D. Bonneau, H. Journel, F. Fellmann, A. Munnich, C. Roux. Service de Génétique et Unité de Recherches sur les Handicaps Génétiques de l'Enfant INSERM U-393, Hôpital des Enfants-Malades (V.C.-D., M.LM., A.M.), Laboratoire de Biologie et d'Embryologie, Hôpital Saint-Antoine, Paris (C.W., C.R.), Service de Pédiatrie, Hôpital Le Bocage, Dijon (A.N.), Unité de Génétique, CH de la Miletrie, Poitiers (D.B.), Unité de Génétique, CH Chubert, Vannes (H.J.), Unité de Génétique, Hôpital Saint-Jacques, Besançon (F.F.), France.

The Smith-Lemli-Opitz (SLO) syndrome is an autosomal recessive disorder characterized by facial phenotype with anomalies of limbs and genitalia [Smith et al., J Pediatr 64:210–217, 1964]. Two types of SLO have been recognized, based on clinical course and severity: the classical form (type I) and the acrodysgenital syndrome usually lethal in the first 12 months of life and frequently associated with severe malformations, markedly abnormal external genitalia, and postaxial polydactyly (type II) [Le Merrer et al., J Med Genet 25:88–95, 1988]. The observation of an abnormally low plasma cholesterol with markedly elevated concentrations of 7 dehydrocholesterol (DHC) in type I patients suggested that a genetic defect of cholesterol biosynthesis might be involved in classical SLO, presumably at the level of the Δ7-DHC reductase [Tint et al., Engl Med 330:107–113, 1994; Irons et al., Am J Med Genet 50:347–352, 1994]. Hitherto, however, little was known regarding the clinical homogeneity of classical SLO and the biochemical bases of the lethal acrodysgenital syndrome (type II) remained obscure.

Taking advantage of a series of 7 unreported patients, we describe severe antenatal growth retardation with severe microcephaly, microretrognatism, and major feeding difficulties as consistent anomalies in type I SLO and support the view that this classical form of the disease is a clinically homogeneous condition. In addition, we provide what we believe to be the first evidence of low plasma cholesterol in the lethal acrodysgenital syndrome (type II) and suggest that the two clinical subtypes share the same biochemical mechanism.

Seven unrelated children (6 females, 1 male) of Western European and North African ancestry were included in the study. Minimum criteria for inclusion were (1) facial phenotype of narrow forehead, anteverted nostrils, hypertelorism, micrognatism, cleft or narrow palate, low-set ears and short neck; (2) limb anomalies, ranging from syndactyly of the 2nd–3rd (fingers and) toes to postaxial polydactyly; and (3) genital anomalies, ranging in males from hypospadias and ectopic testes to sexual reversion. Patients fell into two groups based on age of survival (classical, type I: over 12 months; severe, type II: below 12 months). Five patients had type I and two had type II SLO (Table I).

Plasma cholesterol was determined using both conventional calorimetric techniques and gas chromatography-mass spectrometry which also quantifies cholesterol precursors, derivatives and isomeres. Standard and prometaphasic karyotypes were normal in the probands. Low plasma cholesterol with markedly elevated concentrations of 7-DHC and isomeric DHC were present in all 7 patients, regardless of the clinical subtype (Table II). The level of plasma cholesterol did not correlate with the severity of the disease as cholesterol level was subnormal in one of our type II patients (patient 2) but markedly decreased in several cases of type I SLO. No correlation between 7-DHC or isomeric DHC levels and the severity of the disease could be established either. Interestingly, the presence of trienols was detected in all patients, regardless of the severity of the disease. To our knowledge, trienol accumulation has not been previously reported in SLO. Two families sought genetic counselling for the next pregnancy. Prenatal tests based on detection of cholesterol and 7-DHC in amniotic fluids were normal. The pregnancies were continued and the newborns were normal.

In addition to facial, limb and genitalia anomalies, the following clinical changes were consistently observed: severe microcephaly (head circumference below −4 S.D.), severe ante- and postnatal growth retardation (weight below −2 S.D.) and major feeding difficulties (4/7 patients required gastrostomy). Microretrognatism and low-set ears also were consistently present (Table I) but some anomalies were occasionally absent, namely anteverted nostrils, hypertelorism and postaxial polydactyly (Table I). Other malformations were observed, including pyloric stenosis (3/7), heart septal defects (4/7) and cerebral malformations (4/7, see Table I).

The present study reports on low plasma cholesterol and elevated 7-DHC in seven cases of SLO and provides the first evidence of decreased cholesterol in the lethal acrodysgenital syndrome (SLO type II). This observation gives support to the view that, despite major discrepancies in clinical course and severity, type I and type II SLO are closely related conditions. Yet, no correlations between the level of plasma cholesterol (or 7-DHC) and the severity of the disease could be found in our series. The present study also suggests that classical SLO is a clinically homogeneous condition and describes severe microcephaly, micrognatism, major ante and post natal

TABLE I. Clinical Presentation in Seven SLO Patients

	Patients							
	1	2	3	4	5	6	7	Total
Sex caryotype	F 46, XX	F 46, XX	F 46, XX	F 46, XX	F 46, XX	M 46, XY	F 46, XX	1 M/6 F
Age at diagnosis	10 days	16 days	6 years	3 years	2 years	2 years	8 months	10 d–6 yrs
Facial phenotype								
Anteverted nostrils	+	+	+	−	+	+	+	6/7
Hypertelorism	−	+	+	+	−	+	−	4/7
Micrognatia	+	+	+	+	+	+	+	7/7
Cleft palate	−	+	−	−	+	+	−	3/7
Low set ears	+	+	+	+	+	+	+	7/7
Short neck	−	+	+	+	+	+	−	5/7
Antenatal growth retardation	+	+	+	+	+	+	+	7/7
Failure to thrive	−4 S.D.	−4 S.D.	−4 S.D.	−4 S.D.	−4 S.D.	−2 S.D.	−3 S.D.	<−2 S.D.
Microcephaly	−4 S.D.	−4 S.D.	−4 S.D.	−4 S.D.	−4 S.D.	−4 S.D.	−6 S.D.	<−4 S.D.
Psychomotor retardation	Hypotonia	Hypotonia	No walking No speech	Unable to sit	Unable to sit	Unable to sit	Unable to sit	7/7
Genital anomalies	−	Bicorneal uterus	Clitoris hypertrophy	−	−	Cryptorchidism Hypospadias	−	3/7
Limb anomalies	Postaxial polydactyly Syndactyly 2nd–3rd toe	Postaxial polydactyly Syndactyly 2nd–3rd toe	Syndactyly 2nd–3rd toe	Syndactyly 2nd–3rd toe	Postaxial polydactyly Syndactyly 2nd–3rd toe	Syndactyly 2nd–3rd toe	Postaxial polydactyly Syndactyly 2nd–3rd toe	7/7 7/7
Other malformations		Hydrocephaly		Lissencephaly	Agenesis of corpus callosum	Agenesis of corpus callosum		
		Pyloric stenosis	Pyloric stenosis			Pyloric stenosis		7/7
	Atrial septal defect	Patent ductus arteriosus	Ventricular septal defect				Patent ductus arteriosus	
Outcome	Death 1 month	Death 16 days	Feeding difficulties (→ gastrostomy)	Feeding difficulties	Feeding difficulties (→ gastrostomy)	Feeding difficulties (→ gastrostomy)	Feeding difficulties (→ gastrostomy)	Type I: 5 Type II: 2

TABLE II. Plasma Cholesterol and Their Derivatives in Seven SLO Patients

	Patients								
mg/dl	1	2	3	4	5	6	7	Mean patients	Control
Cholesterol	55	120	34	31	32	55	40	< 55	> 120
7-DHC[a]	26	1	147	26	14	83	47	> 1	< 0.05
Iso DHC I	69	31	194	115	70	61	213	> 30	0
Iso DHC II	Traces	0	0	2	25	0	3	$\neq 0$	0
Trienols	+++	+++	+++	+++	+++	+++	+++	+++	0

[a] DHC, dehydrocholesterol.

growth retardation and feeding difficulties as consistent features in the classical (type I) form of the disease.

3

A RODENT MODEL OF SMITH-LEMLI-OPITZ SYNDROME: BM 15.766-INDUCED TERATOGENESIS. D.B. Dehart, L. Lanoue, G.S. Tint, K.K. Sulik. University of North Carolina Birth Defects Center, Chapel Hill, North Carolina. (D.B.D., L.L., R.R.S.), and Veterans Affairs Medical Center, East Orange, New Jersey (G.S.T.).

Offspring of rats treated with the cholesterol synthesis inhibitor BM 15.766 present with major malformations, including forebrain and midfacial deficiencies. BM 15.766 interferes with the step in cholesterol biosynthesis involving conversion of 7-dehydrocholesterol to cholesterol, the biosynthetic step that also appears to be abnormal in children with Smith-Lemli-Opitz syndrome (SLO). Administration of 300 mg/kg of this inhibitor to pregnant rats for 4 successive days yields maternal serum cholesterol levels that fall, by 6–7 days following treatment initiation, below 15 mg/dl. As with previous teratology investigations conducted by Roux and colleagues involving a similar inhibitor, AY 9944, our studies have shown that malformations that fall within the spectrum of holoprosencephaly are induced when treatment is initiated on gestational day 4. Histological and scanning electron microscopic analyses of these gestational day 11 and 12 embryos illustrate abnormalities in selected cell populations at the rim (alar plate) of the cranial neural folds. This cell population apparently includes premigratory neural crest cells. The affected cells are abnormally shaped, having apparently lost their cell contacts and assuming a spherical configuration. Vital staining has not provided evidence of excessive cell death at these early stages. Treatment initiation on gestational day 6 yields a different pattern of malformation, with abnormal distention of the hindbrain representing a prominent feature as observed on gestational day 13. Additionally, micrognathia is present in affected embryos. Excessive fluid accumulation in the embryos following this later treatment time may reflect damage to the yolk sac. Additional studies are in progress that are directed toward analyses of potential malformations in other organ systems including the heart and secondary palate.

ACKNOWLEDGMENTS

This work was supported by NIH-AA08204 and a grant from the Department of Veterans Affairs Research Service.

4

AUTOPSY DIAGNOSIS OF THE SMITH-LEMLI-OPITZ SYNDROME. F. Faes, E.L.I. Vercruysse, G.A.A. Dacremont, A.M.J.F. De Paepe, I.G. Leroy. Departments of Medical Genetics and Pediatrics, Ghent University Medical School, Belgium.

The finding of abnormally large amounts of 7-dehydrocholesterol (7-DHC) and low levels of cholesterol in plasma of patients with the Smith-Lemli-Opitz syndrome (SLOS) [Tint et al., N Engl J Med 330: 107–113, 1994] was significant in several regards. It proved that this syndrome of multiple congenital anomalies and inadequate neuromotor and mental development [Smith et al., J Pediatr 64: 210–217, 1964] is an inborn error of metabolism due to a block in cholesterol biosynthesis because of deficient reduction of 7-DHC to cholesterol by 7-dehydrocholesterol reductase. It reminded biologists of the key structural role of cholesterol in the formation, function and maintenance of plasma and organelle membranes and illustrated the largely unknown pathogenetic influence of its deficiency in organo- and histogenesis. In particular 7-DHC has become an all important diagnostic marker of SLOS t the clinical geneticist allowing early diagnosis and prenatal diagnosis [Rossiter et al., Am J Med Genet 56: 272–275, 1995; Abuelo et al., Am J Med Genet 56: 281–285, 1995] and objective definition of the clinical spectrum [Penchaszadeh, Am J Med Genet 28: 719–721, 1987; Opitz and de la Cruz, Am J Med Genet 50: 326–338, 1994].

Showing that assay of 7-DHC also allows postmortem diagnosis of SLOS and ultimately objective genetic counselling is the sole purpose of this brief communication.

N.D., the fourth child of Caucasian, healthy, nonconsanguineous parents, was born at term following a pregnancy characterized by paucity of fetal movements in the third trimester. Ultrasonographically, polyhydramnios and enlarged lateral cerebral ventricles were found. The couple's first three children were in good health. The newborn infant in poor general condition had a birth weight of 2,900 g. Her length and head circumference were 47 cm and 33 cm, respectively. Multiple congenital anomalies noticed at birth included (Fig. 1): multiple minorfacial anomalies, proptotic eyes and swollen eyelids, bilateral cataract, apparently low-set ears, broad and flat nose with anteverted nares, mild maxillary hypoplasia, highly arched palate and webbed neck. There was bilateral postaxial hexadactyly in the hands, the appendices lacking radiographically ossified parts, and bilateral syndactyly of the 2nd and 3rd toes with hyperconvex nails. The external genitalia were female with prominent clitoris and hypoplastic labia maiora. The child did not acquire any sucking reflex and remained quite flaccid. Refractory to any supporting measure, the infant died at 22 hours of life.

Cytological and biochemical analysis of blood showed no abnormality. The karyotype was 46,XY. The CT-scan of the brain showed markedly enlarged lateral ventricles and agenesis of the corpus callosum. Large adrenal glands and relatively small kidneys were visualised by abdominal sonography.

Autopsy findings included a large ventricular septal defect, open foramen ovale and patent ductus Botalli. The right lung had only 2 lobes, the left lung only one. The kidneys were small (7 and 10 g) without fetal lobulation. The adrenal glands were rather large (6 g each) but markedly autolysed with a conspiciously small medulla. Testes were found in the inguinal canals. Persistent extramedullary hematopoiesis was prominent in the liver. All findings were consistent with the clinical diagnosis of severe expression of SLOS. This hypothesis was confirmed by the biochemical findings in serum.

Cholesterol and its main derivates were assayed in the patient's serum using capillary column gas chromatography-mass spectrometry [Tint et al., N Engl J Med 330: 107–113, 1994]. Cholesterol was 6.7 mg/dl, a value well below 2 S.D. from the mean in control samples. Among the two additional sterols, the most abundant fraction was identified as the precursor 7-dehydrocholesterol (7-DHC). Its serum concentration was markedly elevated to 8 mg/dl, since in 30 control samples, 7-DHC was not

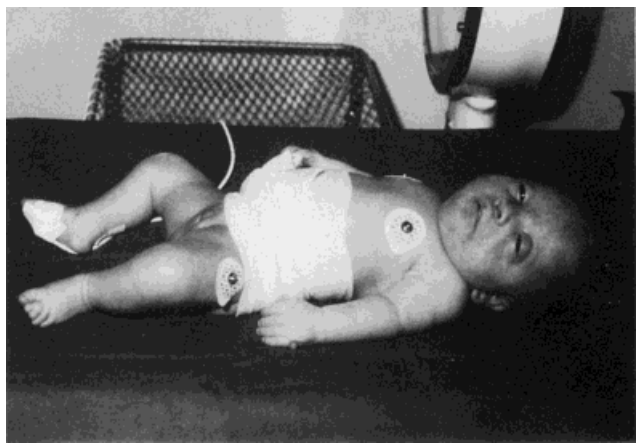

Fig. 1. Patient photographed immediately after death. Note abnormal face, proptotic eyes, flat nose bridge, syndactyly II–III visible only in the left foot, postaxial polydactyly in the hands and female external genitalia.

detectable. The remaining unusual sterol compound was identified as 8-dehydrocholesterol. Serum sterol profiles in the parents were normal.

The clinical diagnosis of SLOS could be made retrospectively because the pediatrician supervising this infant's rapidly fatal clinical course managed to have clinical pictures, radiographs and a CT-scan of the cranium taken and to draw blood samples for karyotyping, routine analysis and for storage and future research purposes. The diagnosis was supported by the autopsy data and prompted the assay of cholesterol and 7-DHC. The results brought about the postmortem confirmation of the diagnosis.

Although the Bialer scoring system did not enable its authors to demonstrate significant intrafamilial variability among published SLOS multiplex families [Bialer et al., Am J Med Genet 78: 723–731, 1987], applied to this infant it yielded a severity score of 14 (cataract: 1; polydactyly: 1; death within 1st week of life: 8; VSD: 2; abnormal lobulation of the lungs: 2) leading to the designation of severe SLOS phenotype. If applied to more patients as a means of quantitative phenotypic assessment, Bialer's scoring system may be helpful in establishing a correlation between clinical effect and course on the one hand and degree of metabolic disturbance on the other. Others have found lower cholesterol levels and a greater proportion of cholesterol metabolites among plasma sterols in patients with the more severe phenotype [Irons et al., Am J Med Genet 50: 347–352, 1994].

Following the discovery of 7-DHC as the marker to faulty cholesterol metabolism and the recognition of SLOS as a monogenic inborn error of morphogenesis [Opitz and de la Cruz, Am J Med Genet 50: 326–338, 1994], any pediatrician or neonatologist attending a malformed infant with rapidly fatal course has one more reason to document carefully the clinical phenotype and to collect blood and tissue samples for diagnosis in particular, in preparation of future effective prenatal diagnostic testing.

5

A "NEW" HERITABLE LIPID ABNORMALITY IN CHILDREN WITH PARTIAL SMITH-LEMLI-OPITZ SYNDROME PHENOTYPE. D. Meschede, Seedorf, U. Seedorf, M. Fobker, P. Miny, U. Flotmann, H.-G. Koch, K. Ullrich, G. Assmann, J. Horst. Institute of Human Genetics, Institute of Atherosclerosis Research and Department of Pediatrics, Westfälische Wilhelms-Universität and St. Franziskus Hospital, Münster, Germany

We have studied the clinical phenotype and plasma lipids in 13 children suspected to have the Smith-Lemli-Opitz syndrome (SLO or RSH syndrome). The study group included 6 females and 7 males aged 2 weeks to 14 years. In all patients we documented the presence or absence of 17 key anomalies defining the SLO syndrome clinically. Plasma lipids were analysed with gas and high performance liquid chromatography (GC, HPLC), time-of-flight secondary-ion mass spectometry (TOF-SIMS), and a spectrophotometric method developed in our laboratory. Three patients were found to have low plasma levels of cholesterol and grossly elevated concentrations of 7-dehydrocholesterol (7-DHC). This sterol pattern, indicating a block in the final step of cholesterol biosynthesis, is pathognomonic for the SLO syndrome [Tint et al., NEJM 330:107, 1994]. The number of key clinical traits present in these three patients was 14, 10 and 14, respectively.

Two patients ("A" and "B") had a different and previously undescribed abnormal sterol pattern upon HPLC analysis. They were positive for 10 and 11 key clinical features of the SLO syndrome, respectively. In addition to peaks representing physiological plasma compounds, both children displayed abnormal absorption peaks with a retention time of 38 (P_{38}) and 39 minutes (P_{39}). We have termed this the type B sterol pattern. A sterol pattern of this type was neither observed in several hundred analyses performed in our laboratory over the past two years nor in 25 normal controls specifically recruited for this study. Interestingly, both parents of patient A and the father of patient B displayed the same pattern on HPLC analysis, but the absorption peaks were smaller than in their affected offspring. The parents of patient A refused further investigations and withdrew from the study. The family of patient B was investigated in more detail by analysing sterol profiles in all 4 grandparents and 4 uncles and aunts. In the maternal line, all tested individuals had normal sterol patterns. On the paternal side, however, the patient's father, one aunt, 2 uncles and the grandmother displayed the type B pattern. None of these individuals had any notable health problems. There was no discernible correlation between the height of the P_{38}/P_{39} peaks and the clinical status of the individual. One paternal uncle had peaks of the same height as the affected child. Inheritance of the type B sterol pattern could be autosomal dominant in this family, with marked intrafamilial variability.

We currently attempt to elucidate the exact chemical structure of the plasma compounds represented by the abnormal P_{38}/P_{39} peaks. So far, the clinical and pathophysiological significance of the type B sterol pattern in children with partial SLO syndrome phenotype remains unclear. This lipid abnormality might be an indicator for a metabolic disorder distinct from the enzymatic defect in SLO syndrome. However, we still can not exclude that the type B pattern is a spurious finding causally unrelated to the clinical presentation in our patients.

6

7-DEHYDROCHOLESTEROL AND CHOLESTEROL BIOSYNTHESIS IN CULTURED CELLS. G.S. Tint, A. Honda, M. Honda, A.K. Batta, T. Chen, A.K. Batta, S. Shefer, G. Xu, G. Salen. Department of Veterans Affairs Medical Center, East Orange, and UMD-New Jersey Medical School, Newark, New Jersey

The Smith-Lemli-Opitz (SLO) syndrome is associated with reduced plasma and tissue levels of cholesterol together with markedly elevated concentrations of 7-dehydrocholesterol (cholesta-5,7-dien-3β-ol, 7-DHC) and its epimer 8-dehydrocholesterol (cholesta-5,8-dien-3β-ol, 8-DHC) [Irons et al., Lancet 341:1414, 1993; Tint et al., N Engl J Med 330:107, 1994]. These findings suggest that the enzyme 3β-hydroxysteroid Δ^7-reductase (7-DHC Δ^7-reductase) which catalyses the transformation of 7-DHC to cholesterol (the final step in cholesterol biosynthesis) is defective.

To assay the functionality of 7-DHC Δ^7 reductase, [^{3}H]-lathosterol (cholest-7-en-3β-ol), the immediate precursor of 7-DHC, was incubated for 24 hours with cultured fibroblasts or amniocytes from 15 affected subjects (7 type I, 8 type II), 14 parents and 8 controls. Sterols were extracted and purified and the percentage of recovered label found in cholesterol and 7-DHC determined [Honda et al., J Lipid Res, 36:1595, 1995]. To test the viability of that portion of the cholesterol biosynthetic pathway leading to the formation of 7-DHC, the identical procedure was carried out with [^{3}H]-mevalonolactone rather than lathosterol in the incubation mixture. In addition, because we had noted markedly reduced activity of hydroxymethylglutaryl coenzyme A reductase (HMG-CoAR), the rate-controlling enzyme for cholesterol biosynthesis, in rats after 3 days administration of the 7-dehydrocholesterol Δ^7-reductase inhibitor BM 15.766 [Xu et al., J Clin Invest, 95:76, 1995] we investigated the effects of 7-dehydrocholesterol on cholesterol biosynthesis in affected and control cultured fibroblasts.

The conversion of radiolabeled lathosterol to cholesterol and 7-DHC is summarized in Table I as mean ± S.E.M.

Labeled mevalonate was efficiently converted to cholesterol by parents' fibroblasts (65% of label) but poorly transformed by homozygous cells (0.5% and 0.6%). In contrast, in affected fibroblasts 34% and 20%, respectively, of ^{3}H was recovered as 7-DHC compared to only 2.3% found as 7-DHC in the heterozygous cells.

HMG-CoAR activity in control (n = 14), type I SLO (n = 7) and type II SLO (n = 5) fibroblasts grown in medium with fetal bovine serum were within normal limits at 12 ± 1, 12 ± 3 and 17 ± 4 pmol/min/mg protein, respectively, and the enzyme upregulated normally to 80 ± 12, 77 ± 15 and 74 ± 7 pmol/min/min, respectively, after 24 hours incubation in delipidated medium. When 7-DHC was added to affected cells grown in delipidated medium, HMGCoAR was suppressed in a dose-dependent manner decreasing to about 4% of baseline activity when the concentration of added 7-DHC was increased to 20 μg/ml. In contrast, adding cholesterol at the same concentration reduced HMG-CoAR activity only to 60% of baseline.

The defect in the Smith-Lemli-Opitz syndrome is, indeed, markedly reduced conversion of 7-DHC to cholesterol which is probably expressed in all tissues, and the block in cholesterol biosynthesis appears to be significantly more severe in the most seriously affected subjects. The remainder of the pathway, up to the step in which the C-7 double bond of 7-DHC is reduced to yield cholesterol, appears to be unaffected. HMG-CoA reductase, the rate-controlling enzyme for cholesterol biosynthesis, functions normally in affected fibroblasts and is upregulated properly after incubation in delipidated medium. However, 7-DHC appears to be a potent inhibitor of HMG-CoAR. The latter result suggests that low total sterol levels in the syndrome may be further exacerbated by high plasma and tissue concentrations of 7-DHC.

ACKNOWLEDGMENTS

This work was supported by grants from Research Service, Department of Veterans Affairs and U.S. Public Health Service grants HD-31932, DK-18707, HL-17818.

TABLE I. Conversion of Radiolabeled Lathosterol to 7-DHC and Cholesterol

		% of recovered label found in	
Patients	n	7-DHC	Cholesterol
SLO Type I	7	50 ± 1	1.1 ± 0.2*
SLO Type II	8	45 ± 4	0.03 ± 0.03*,**
Parents	14	7.9 ± 1.2***	59 ± 2
Controls	8	3.2 ± 0.7	64 ± 3

* $P < 0.001$ vs. parents and controls.
** $P < 0.001$ vs. Type I.
*** $P < 0.01$ vs. controls.